Article

The prevalence of bacterial contamination of surgical cold sterile solutions from community companion animal veterinary practices in southern Ontario

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Abstract — Surgical cold sterile solutions are commonly used in veterinary practice, yet sterility cannot be verified under practical clinical conditions. Surgical cold sterile solutions were sampled and bacteria, including opportunistic pathogens, were recovered from 13% of the sampled solutions. Attempts to sterilize surgical instruments with cold sterile solutions should be avoided.

Résumé — Prévalence de la contamination bactérienne des solutions chirurgicales stériles froides dans les pratiques vétérinaires communautaires pour animaux de compagnie du Sud de l'Ontario. Les solutions chirurgicales stériles froides sont couramment utilisées en pratique vétérinaire, pourtant la stérilité ne peut pas être vérifiée dans des conditions cliniques pratiques. Des échantillons ont été prélevés des solutions chirurgicales stériles froides et des bactéries, incluant des agents pathogènes opportunistes, ont été récupérées dans 13 % des échantillons de solutions prélevés. Il faut éviter de tenter de stériliser les instruments chirurgicaux à l'aide de solutions stériles froides.

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Introduction

Sterilization is a standard practice for surgical instruments and is typically achieved by steam under pressure (autoclave). For instruments that cannot withstand steam sterilization, such as endoscopes, there are other sterilization methods including peroxide vapor, ethylene oxide gas, and chemical sterilant solutions (commonly called "cold sterilization"). Cold sterilization involves immersion of items in a sterilant solution, such as glutaraldehyde or alcohol, for a predetermined period of time. Some cold sterile solutions and protocols are able to achieve sterilization or high level disinfection; however, not all disinfectants and practices can effectively eliminate all microbial contaminants.

There are no published data describing the use of surgical cold sterile solutions in veterinary medicine; however, surgical cold sterile solutions are used in community veterinary practices to sterilize dental instruments, suture needles and suture material, and surgical instruments for minor surgical procedures. These surgical procedures include clean surgical procedures (such as, lump removal), clean contaminated surgical procedures (for example, feline castration, feline onychectomy, and wound debridement), and dirty surgeries (such as, lancing an abscess). In addition, instruments may be obtained from a surgical cold sterile solution to replace surgical instruments from a sterile surgical pack that may have become contaminated during sterile surgical procedures.

There are numerous concerns regarding the use of cold sterile solutions, particularly for surgical instruments that are used in sterile body sites. Sterilization using cold sterile solutions is a lengthy procedure; under normal clinical conditions, it takes approximately 10 h for an instrument to become sterile (1). Also, cold sterile solutions can be easily contaminated through the introduction of particulate or organic matter. This can occur when cold sterile solutions are open and exposed to air, through the addition of improperly cleaned instruments, and the retrieval of instruments using contaminated objects, including fingers. Furthermore, cold sterile solutions need to be managed properly so that alterations in dilution, pH, temperature, contact time, organic load, and frequency of use do not reduce the effectiveness of these solutions.

This study aimed to determine the prevalence of surgical cold sterile solutions in community companion animal veterinary practices and the prevalence of bacterial contamination of these solutions. In addition, this study examined associations between bacterial recovery from cold sterile solutions and specific practice demographics.

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Materials and methods

Clinics were selected and sampling was done during the summer of 2005. Veterinary hospitals in southern Ontario licensed as companion animal hospitals or offices, including those with additional licenses for food animal or equine hospital or mobile (mixed-animal practices), by the College of Veterinarians of Ontario in 2005 were eligible for recruitment. A recruitment letter was mailed to these practices (n = 766) describing the study objectives. Practices willing to participate were asked to respond by mail, fax, or telephone with a completed practice-demographic survey. Based on previous experience in community-based research in companion veterinary practices in southern Ontario, we sought to enroll 100 veterinary practices. This also provided sufficient statistical power to detect bacterial contamination at a prevalence of 10%.

Cold sterile solutions in the surgery room of the study practices were sampled. Samples were not collected from cold sterile solutions in other areas of the practice (such as, treatment rooms, examination rooms); samples were not collected from cold sterile solutions used exclusively for instruments used in dental procedures. Data were not collected on the type of chemical sterilants used in the cold sterile solutions. The solutions were sampled (approximately 1 to 3 mL) aseptically using a syringe. Approximately 16 to 24 h after sampling, 100 μL aliquots of cold sterile solution were streaked onto 2 blood agar plates. One plate was incubated aerobically at 35°C and the other anaerobically at 37°C. Isolates were identified using Gram stain and appropriate biochemical tests followed by use of commercial identification kits for *Staphylococcus* spp. (API Staph, BioMerieux Canada, St. Laurent, Quebec), *Streptococcus* spp. (API 20 Strep, BioMerieux Canada) or Gram negatives (BBL Enterotube II, Becton, Dickinson and Co., Franklin Lakes, New Jersey, USA).

Descriptive statistics were used to determine the prevalence of cold sterile solutions in veterinary practices, the prevalence of specific bacteria recovered, and overall prevalence of bacterial recovery. All variables were categorical and Fisher's exact test was used to determine associations between overall bacterial recovery and practice demographics: practice type (companion animal or mixed animal), number of hospitalized patients per day, number of appointments per day, number of staff and presence of a "Standard Operating Procedure" for sterilization practices. Statistical significance was determined using a P-value ≤ 0.05 and a 95% confidence interval (CI) for the odds ratio (OR) that did not include the null. Statistical analysis was performed using Microsoft Excel 2003 (Microsoft Corporation, Redmond, Washington, USA) and Intercooled Stata 10.0 software (StataCorp, College Station, Texas, USA).

Results

One hundred and twenty-one clinics responded with interest to the recruitment letter (response rate 16%). Twelve of these practices could not be sampled because of time limitations and 8 practices were out of the geographic sampling region. Therefore from the initial 121 clinics that responded with interest, the study population of 101 veterinary practices was enrolled. Of the 101 practices, 90 practices were companion animal, 10 were mixed animal, and 1 practice treated primarily exotic animals.

Table 1. The prevalence of bacterial growth from surgical cold sterile solutions (n = 101) from community companion animal veterinary practices (n = 101) in southern Ontario

Organism	Prevalence (%) (95% CI)
Aerobes	
Mannheimia haemolytica	3 (0.6, 8)
Burkholderia cepacia	3 (0.6, 8)
Shigella species	1 (0.02, 5)
Coagulase negative *Staphylococcus* species	1 (0.02, 5)
Serratia marcescens	1 (0.02, 5)
Citrobacter freundii	1 (0.02, 5)
Acinetobacter lwoffii	1 (0.02, 5)
Anaerobes	
Bacillus species	3 (0.6, 8)
Clostridium fallax	1 (0.02, 5)

CI — confidence interval.

The prevalence (and one-sided 97.5% CI) of use of surgical cold sterile solutions in community veterinary practices was 100% (96%, 100%), and each practice had only 1 surgical cold sterile solution. The prevalence (and 95% CI) of aerobes and anaerobes recovered from surgical cold sterile solutions was 11% (6%, 19%) and 5% (2%, 11%), respectively. When combined, 13% (7%, 21%) of the cold sterile solutions yielded bacterial growth (aerobic or anaerobic), including veterinary and human opportunistic pathogens (Table 1). There were no significant associations (P-values > 0.5) between bacterial recovery and the tested practice demographics.

Discussion

In 1968, Earle H. Spaulding (2) devised a classification system to guide hospital disinfection and sterilization based on the risk of infection to the human patient. This system classified any object that entered sterile tissue or the vascular system as "critical." Autoclaving was the recommended method of sterilization for "critical items." The recommended use of chemical sterilants or cold sterile solutions was limited to instruments where sterilization by other means was unsuitable. The same principles are still recommended (1), and cold sterile solutions are not recommended for instruments used in sterile surgical procedures (3) because sterility cannot, for practical purposes, be verified when using cold sterile solutions. In other sterilization methods, objective evaluations of sterility can be easily performed, such as the use of indicator strips and biological indicators for autoclaves (1).

The observed high prevalence of bacterial recovery, including some opportunistic pathogens, from surgical cold sterile solutions was striking. This is especially remarkable since methods were not employed to neutralize the chemical sterilants in the cold sterile solutions prior to bacterial isolation. Furthermore, the time from sample collection to culture was greater than 10 h, and this time lag should have been sufficient for the solution to become sterile if it had been contaminated, even by normal procedures such as the addition of clean instruments, at the time of sampling. This suggests that factors, such as

inappropriate dilution or pH or contamination with particulate or organic matter, may have reduced the efficacy of the sampled surgical cold sterile solutions. This time-lag, however, may have resulted in the elimination of some bacteria that are typically susceptible to disinfection, for example, gram-positive bacteria. Consequently, we may have underestimated the prevalence of bacterial contamination and the diversity of viable pathogens in surgical cold sterile solutions.

A diverse group of bacteria were recovered; isolation of sporeformers (*Bacillus* and *Clostridium* spp.) was not surprising since bacterial spores are highly resistant to disinfectants (1). Similarly, *Serratia* spp. can be tolerant to disinfectants and have a propensity for colonization of solutions (4). Yet, the recovery of important opportunistic pathogens, such as *Staphylococcus* and *Acinetobacter* spp., is a concern because these organisms are generally susceptible to disinfectants and chemical sterilants. The origin (human or animal) of the bacterial contaminants is unknown. Since some of these organisms are components of human skin (5) and fecal (6) flora, contamination may have occurred through mishandling or inadequate hand hygiene. However, some of the bacterial isolates could be of animal origin (for example, *Burkholderia cepacia*) (7) and may have been introduced into the solution through improperly cleaned instruments.

Some organisms recovered in this study have been associated with severe infections in dogs such as septicemia (8,9) and surgical site infections (10) including an infection of a surgical implant (11). Therefore, the bacteria recovered from the surgical cold sterile solutions pose a potential health risk to surgical patients. In addition, such infections could contribute to the overall epidemiology of opportunistic bacteria in the community through zoonotic transmission of an animal infection to a person. The magnitude of these potential risks is unknown as there is a general lack of information on the epidemiology of hospital associated infections, including SSI in companion animal veterinary medicine and the risk posed to animal and human health. Reducing these risks is achieved by adherence to aseptic principles. The results of this study indicate that the use of surgical instruments from a cold sterile solution contravenes aseptic principles. In addition to the possible negative animal and human health consequences associated with this contravention, there could also be legal and professional consequences for veterinarians.

Minimizing adverse surgical events in companion animal veterinary medicine is one goal of an effective infection control program. Strict adherence to aseptic surgical principles is imperative in order to achieve this goal. The use of cold sterile solutions should be limited in veterinary medicine and sterilization of instruments or equipment for sterile procedures by these solutions should be avoided.

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